

FORM PTO-1390  
(REV. 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

GJE-63

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

UNKNOWN 09/806548

INTERNATIONAL APPLICATION NO.  
PCT/GB99/03290INTERNATIONAL FILING DATE  
05 OCTOBER 1999PRIORITY DATE CLAIMED  
06 OCTOBER 1998TITLE OF INVENTION  
ABSORBENT MEDIUM AND ITS USE IN PURIFYING DNAAPPLICANT(S) FOR DO/EO/US  
ANGUS, Katherine L.; HUTTON, David A.; NOEL, Robert J.; TAYLOR, Linda

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☒ has been communicated by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a. ☐ is attached hereto.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ have been communicated by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (unsigned).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

## Items 11 to 20 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 37 CFR 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information: PTO Form 1449 and references listed thereon  
International Search Report

COPY

U.S. APPLICATION NO. <b>09/806548</b> UNKNOWN		INTERNATIONAL APPLICATION NO. PCT/GB99/03290		ATTORNEY'S DOCKET NUMBER GJE-63	
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21. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. .... <b>\$1000.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$860.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$710.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$690.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b>  <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS PTO USE ONLY</b>          <div style="border: 1px solid black; width: 100px; height: 100px; margin: 0 auto;"></div>	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				<b>\$860.00</b>	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$
Total claims	20 - 20 =	0	x \$18.00	\$0.00
Independent claims	2 - 3 =	0	x \$80.00	\$0.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ \$270.00
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$860.00</b>
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+
<b>SUBTOTAL =</b>				<b>\$860.00</b>
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				
<b>TOTAL NATIONAL FEE =</b>				<b>\$860.00</b>
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +				
<b>TOTAL FEES ENCLOSED =</b>				<b>\$860.00</b>

	Amount to be refunded: \$
	charged: \$

a. ☐ A check in the amount of \$ \_\_\_\_\_ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 19-0065 in the amount of \$ 860.00 to cover the above fees.  
 A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any  
 overpayment to Deposit Account No. 19-0065. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card  
 information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR  
 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

CORRESPONDENCE ADDRESS:


CUSTOMER NUMBER <b>23,557</b>	March 30, 2001 DATE	<div style="text-align: center;">           SIGNATURE       </div> Frank C. Eisenschenk, Ph.D. NAME 45,332 REGISTRATION NUMBER
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09/806548

JC08 Rec'd PCT/PTO 30 MAR 2001

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail in an envelope addressed to Box PCT, the Assistant Commissioner for Patents, Washington, D.C. 20231 on March 30, 2001.

PRELIMINARY AMENDMENT  
Patent Application  
Docket No. GJE-63

  
Frank C. Eisenschenk, Ph.D., Patent Attorney

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Katherine Louise Angus, David Alan Hutton, Robert John Noel,  
Linda Taylor  
Docket No. : GJE-63  
For : ADSORBENT MEDIUM AND ITS USE IN PURIFYING DNA

Box PCT  
Assistant Commissioner for Patents  
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

It is respectfully requested that the above-identified patent application be amended as follows:

In the Specification

After page 7: Please insert as new page 8 the attached Abstract of the Disclosure.

In the Claims

Please substitute the following claims:

Claim 3 (Amended):

A medium according to claim 1, which has a water retention value of greater than 6 ml/g.

Claim 4 (Amended):

A medium according to claim 1, wherein the sponge material is a naturally-occurring polymer.

Claim 6 (Amended):

A medium according to claim 1, wherein the functional groups bind DNA.

Claim 8 (Amended):

A medium according to claim 1, wherein the particles are 0.5 to 10 mm in size.

Claim 9 (Amended):

A medium according to claim 1, capable of retaining species having a molecular weight of at least one million Daltons.

Claim 10 (Amended):

A medium according to claim 1, wherein the sponge material is cross-linked.

Claim 11 (Amended):

A method for purifying DNA in an aqueous sample, which comprises passing the sample through a medium according to claim 1.

Please add the following new claims 12-20:

12. A medium according to claim 1, wherein the particles are of heterogeneous particle size and are obtained by chopping a larger block of the cellulosic sponge material.

13. A medium according to claim 8, wherein the particles are of heterogeneous particle size and are obtained by chopping a larger block of the cellulosic sponge material.

14. A medium according to claim 2, wherein the sponge material is free of fibrous reinforcement and comprises particles of heterogeneous particle size obtained by chopping a larger block of the cellulosic sponge material.

15. A medium according to claim 14, wherein the particles are cross-linked.

16. A medium according to claim 15, wherein the particles are 0.5 to 10 mm in size.

17. A medium according to claim 16 having a water retention value of greater than 6 ml/g.

18. A medium according to claim 3, wherein said sponge material comprises particles of cellulosic sponge material carrying functional groups and wherein said particles are cross linked.

19. A medium according to claim 18, wherein said particles are of heterogeneous size and are obtained by chopping a larger block of cellulosic sponge material.

20. A medium according to claim 19, wherein said particles are 0.5 to 10mm in size.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D.

Patent Attorney

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FCE/jaj

Attachment: Abstract of the Disclosure  
Marked-Up Version of Amended Claims

### Abstract of the Disclosure

An adsorbent medium comprises particles of a cellulosic sponge material carrying functional groups. This medium is especially useful for purifying DNA in an aqueous sample.

MARKED-UP VERSION OF AMENDED CLAIMS

Claim 3 (Amended):

A medium according to claim 1 ~~or claim 2~~, which has a water retention value of greater than 6 ml/g.

Claim 4 (Amended):

A medium according to claim 1 ~~any preceding claim~~, wherein the sponge material is a naturally-occurring polymer.

Claim 6 (Amended):

A medium according to claim 1 ~~any preceding claim~~, wherein the functional groups bind DNA.

Claim 8 (Amended):

A medium according to claim 1 ~~any preceding claim~~, wherein the particles are 0.5 to 10 mm in size.

Claim 9 (Amended):

A medium according to claim 1 ~~any preceding claim~~, capable of retaining species having a molecular weight of at least one million Daltons.

Claim 10 (Amended):

A medium according to claim 1 ~~any preceding claim~~, wherein the sponge material is cross-linked.

Claim 11 (Amended):

A method for purifying DNA in an aqueous sample, which comprises passing the sample through a medium according to claim 1 ~~any preceding claim~~.



## ADSORBENT MEDIUM AND ITS USE IN PURIFYING DNA

Field of the Invention

This invention relates to an adsorbent medium and to its use in retaining large molecular species, e.g. in the purification of DNA.

Background of the Invention

DEAE (diethylaminoethane) has been used to derivatise Sepharase or Sephadex beads to provide an anion exchange resin, having quaternary ammonium groups. This is available from Pharmacia as DEAE-streamline. It is used as an anticholesteremic.

Cellulosic sponge materials have been used in various forms as the basis of an adsorbent medium, typically after the introduction of ion-exchange groups. For example, GB-A-1226448 discloses the introduction of cross-linking residues into regenerated cellulose, together with or followed by the introduction of ion-exchange groups. The resultant medium can be used, packed in a column. Various physical forms are disclosed.

WO-A-9117830 discloses an adsorbent medium (HVFM) prepared by cross-linking a flexible hydrophilic sponge that contains fibrous, hydrophilic reinforcement, and introducing functional groups. The cross-linking is controlled so that the resulting sponge has a water retention value of 2 to 6. It is proposed that this medium is suitable for the isolation or separation of macromolecules such as proteins, while retaining mechanical strength. However, in addition to its low water retention value, this medium has poor flow properties.

A particular area in which an efficient, commercial process is required, for purifying samples, is in the preparation of pharmaceutical grade plasmid DNA for gene therapy. Although many processes have been developed for the large-scale production of recombinant plasmid DNA, e.g. using *E. coli* as the host cell, purification of the product is necessary before the functional gene can be used, i.e.

for expression in somatic tissues with the intention of selectively correcting or modulating disease conditions.

Purification of plasmid DNA has traditionally been on a very small scale for research purposes. Purification of plasmid DNA for preclinical toxicology, human clinical trials, and ultimately for an approved pharmaceutical indication, requires a process that reproducibly meets all the quality and regulatory standards and can be used to purify large quantities of the material economically.

The standard method for plasmid DNA purification by molecular biologists has been by caesium chloride/ethidium bromide ultracentrifugation. This method is unacceptable for the production of clinical materials, because it uses mutagenic reagents and is unscalable.

Alternative methods for the purification of plasmid DNA have been developed using a combination of chromatography techniques. Problems associated with such techniques have been low capacity of the adsorbents for plasmid DNA, denaturation and breakdown of the DNA molecule, and losses due to filtration of the feedstock containing plasmid DNA. Problems associated with filtration have been resolved by using the adsorbents in fluidised bed mode, but this requires specially designed hardware for large-scale applications.

#### Summary of the Invention

This invention is based on the discovery that an adsorbent medium comprising particles of a cellulose sponge material carrying functional groups, can be effectively used to retain species having molecular weight of at least one million Daltons, e.g. viruses and DNA. In particular, it has been found that an effective adsorbent can be prepared from readily-available sponge material, after removal of fibrous reinforcement.

The new porous matrix has high capacity for plasmid DNA, and can be used in a packed bed with unfiltered feedstock. It can be used in any type of chromatographic

technique, including ion-exchange, and also affinity and hydrophobic interaction.

#### Description of the Invention

5 The cellulosic sponge material, from which the novel medium is derived, is typically a naturally-occurring polymer such as cellulose or agarose.

Cellulosic sponge materials are commercially available, including hydrophilic, fibrous reinforcement. For the purposes of the present invention, this  
10 reinforcement can be removed, or at least substantially removed. The water retention of the medium is generally higher than that described in WO-A-9117830, and is typically greater than 6 ml/g, e.g. up to 10 or 11 ml/g.

The material used in the invention is preferably  
15 cross-linked. This may be achieved by known procedures. Similarly, the introduction of functional groups may be achieved in known manner, e.g. as described in WO-A-9117830. Suitable ion-exchange groups are known. It is preferred that the functional groups should bind DNA. They  
20 may be derived from tertiary amines such as diethylaminoethane (DEAE), or quaternary amines.

The adsorbed medium of this invention may be made into particles of suitable size by any appropriate technique, e.g. chopping. The size of the particles may be  
25 heterogeneous; it is not necessary to control its uniformity. The particles will usually be at least 0.1 mm in size, e.g. up to 10 mm or more; a preferred particle size is 0.5 or 1 to 10 mm.

In an illustrative embodiment of the invention, an  
30 open structure fibrous polymer adsorbent (HVFM) has been developed for the purification of plasmid DNA. In order to demonstrate its suitability, the material was converted into a weak anion-exchanger (DEAE-HVFM) and used to purify plasmid DNA from unfiltered feedstock.

35 Particulate HVFM was derivatised, up to a level of 1933  $\mu$ moles per g dry weight, with diethylaminoethane (DEAE). The characteristics of particulate DEAE-HVFM for

the purification of plasmid DNA from crude feedstock were determined. The dynamic capacity of DEAE-HVFM for plasmid DNA was  $>1500 \mu\text{g}.\text{ml}^{-1}$  and  $1350 \mu\text{g}.\text{ml}^{-1}$  at  $88 \text{ cm}.\text{h}^{-1}$  (30 column volumes per hour) and  $175 \text{ cm}.\text{h}^{-1}$  (60 column volumes per hour), respectively.

Analysis of the purified plasmid DNA was carried out by various methods: it contained no detectable amount of RNase and was not damaged by this purification process. The pressure/flow properties of particulate DEAE-HVFM showed that a flow rate of 116 column volumes per hour can be achieved at a pressure of 2.2 bar, across a column having a bed height of 4 cm and which is 2.5 cm in diameter.

The water retention value of the particulate DEAE-HVFM is approximately 10 ml/g. A water retention value of 2 to 6 is quoted for HVFM with a uniformly distributed fibrous reinforcement (WO-A-9117830).

The following Examples illustrate the invention.

White HVFM Cloth (18 x 20cm) was washed in tap water at  $50^{\circ}\text{C}$  followed by RO  $\text{H}_2\text{O}$  and rolled dry. The HVFM was cut into pieces (approximately 2cm square) and the 'nylon' scrim reinforcement was removed. The HVFM pieces were covered with water in a plastic beaker and homogenised using a hand-held Kenwood Blender for approximately 2 minutes. Homogenised HVFM was poured into a Pharmacia XK-50 column and squeezed dry using the plunger of the column.

430ml  $\text{H}_2\text{O}$  and 3.5ml 1,3-dichloropropanol were mixed and added to the homogenised HVFM in a 500ml glass beaker and stirred gently to mix. The beaker was covered with non-PVC cling film (four sheets) and 1 layer of aluminium foil and incubated for 1h at  $60^{\circ}\text{C}$  in an oven. The reagents were poured off and the sponge washed and dried under vacuum suction and then by plunging in an XK-50 column.

55.4g DEAE was dissolved in 122ml RO  $\text{H}_2\text{O}$  and poured onto the cross-linked HVFM in a 500ml glass beaker and gently mixed. 250ml 5M NaOH was poured evenly onto the HVFM and mixed gently. The beaker was covered in 4 sheets of

non-PVC cling film and one sheet of aluminium foil and incubated for 1h. at 60°C in an oven. The reagents were poured off and the sponge dried under vacuum suction. Fresh solutions of 45% (w/v) DEAE and 5M NaOH were added to the sponge as above and incubated again at 60°C. The reagents were poured off and the sponge washed extensively under vacuum suction, followed by squeezing dry in an XK-50 column. This procedure gave HVFM derivatised with DEAE.

A 1ml Omni-fit column (ID 0.66cm) was prepared by hydrating approximately 1g wet weight DEAE-HVFM with 20mM Tris pH 7.5 and packing it into the column using the column plunger. The column was then equilibrated with 0.2M NaCl (adjusted to pH 11.0 with NaOH) at 0.5ml.min<sup>-1</sup>. 35 ml 12mM HCl was then washed through the column at 0.5ml.min<sup>-1</sup>. 1ml fractions were collected and the pH of each fraction measured. The column was then unpacked and the DEAE-HVFM dried to constant weight at 50°C in an oven. A graph of pH against mmoles of HCl applied to the column was plotted and the point at which the pH dropped sharply to approximately pH 3.0 was used to calculate the level to which the sponge had been derivatized with DEAE.

In summary, white sponge cloth that had been filleted of its reinforcing mesh and also homogenised was derivatized. The level of derivatization achieved was 1933µmoles DEAE per g dry weight.

The capacity of DEAE-HVFM (homogenised) was at least 5 times as much as that estimated for DEAE-streamline, i.e. 150-300µg.mg<sup>-1</sup>. With increased capacity for plasmid DNA, it might be expected that the DEAE-HVFM may also have increased capacity for RNA and protein; therefore, the levels of these residuals between the two matrices was compared. No detectable RNAase, the predominant protein contaminant in the feedstock, was found in the 0.5M and 1.0M elutions of either matrix after NuPAGE electrophoresis and silver staining. RNAase could be detected in the breakthrough and the 20mM Tris washes from both matrices. Gel filtration on Sephacryl S-500 showed that low molecular

weight contaminants were present in the 1.0M elutions from each of the columns. SYBR green II staining of samples of the 1.0M elutions run on 1.0% agarose gels suggested that these contaminants are digested RNA, and the proportion of material in the plasmid DNA peak from the DEAE-HVFM column was greater than that from the DEAE-Streamline column (5.4% cf 1.7%), i.e. a three-fold increase in plasmid DNA purity relative to RNA.

Agarose gel electrophoresis of plasmid DNA eluted from both DEAE-HVFM and DEAE-streamline demonstrated that the plasmid was not damaged by binding and elution from the column. In contrast, silica-based matrices such as that found in the Plasmix purification kit nick the DNA and produce more of the open circular form.

The pressure/flow characteristics of particulate DEAE-HVFM were compared with those of DEAE-HVFM cloth with reinforcement and DEAE-HVFM 'natural' block. For all matrices, the flow rate increased with decreasing bed height. However, when the matrices were compared at a fixed bed height, the particulate DEAE-HVFM had better flow properties. For example, particulate DEAE-HVFM achieved a flow rate of 116 column volumes per hour at a pressure of 2.2 Bar, across a column of dimensions 4.0cm bed height and 2.5cm diameter; the flow rates for DEAE-HVFM reinforced cloth and DEAE-HVFM 'natural' block were 79 and 85 column volumes per hour respectively.

The DEAE-HVFM was also found to have better pressure/flow characteristics than Whatman Express ion-exchangers Q and DEAE.

CLAIMS

1. An adsorbent medium comprising particles of a cellulosic sponge material carrying functional groups.
2. A medium according to claim 1, wherein the sponge material is free of fibrous reinforcement.
3. A medium according to claim 1 or claim 2, which has a water retention value of greater than 6 ml/g.
4. A medium according to any preceding claim, wherein the sponge material is a naturally-occurring polymer.
5. A medium according to claim 4, wherein the polymer is cellulose or agarose.
6. A medium according to any preceding claim, wherein the functional groups bind DNA.
7. A medium according to claim 6, wherein the functional groups are derived from DEAE.
8. A medium according to any preceding claim, wherein the particles are 0.5 to 10 mm in size.
9. A medium according to any preceding claim, capable of retaining species having a molecular weight of at least one million Daltons.
10. A medium according to any preceding claim, wherein the sponge material is cross-linked.
11. A method for purifying DNA in an aqueous sample, which comprises passing the sample through a medium according to any preceding claim.

12. A medium according to any of claims 1 to 10, wherein the particles are of heterogeneous particle size and are obtainable by chopping a larger block of the cellulosic sponge material.



DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of subject matter which is claimed and for which a patent is sought on an invention entitled  
**ADSORBENT MEDIUM AND ITS USE IN PURIFYING DNA**

the specification of which ☐ is attached hereto or

☒ was filed on **05 OCT 1999** as United States Application Number or PCT International Application Number **PCT/GB99/03290** and was amended on **15 NOV 2000** (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for a patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application Number(s)	Country	Foreign Filing Date	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
9821783.9	GB	06 OCT 1998	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: **David R. Saliwanchik, Reg. 31,794; Jeff Lloyd, Reg. 35,589; Doran R. Pace, Reg. 38,261; Christine Q. McLeod, Reg. 36,213; Jay M. Sanders, Reg. 39,355; James S. Parker, Reg. 40,119 and Jean E. Kyle, Reg. 36,987; Frank C. Eisenschenk, Reg. 45,332; Seth M. Blum. Reg. 45,489**

9

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C 1001 and that such willful false statements may jeopardise the validity of the application or any patent issued thereon.

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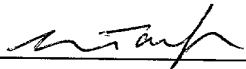
Post Office address c/o Bioprocessing Ltd., Unit 31, No. 1 Industrial Estate, Medomsley Road, Consett, Co. Durham DH8 6SZ, United Kingdom

Country of Citizenship United Kingdom Date of signature \_\_\_\_\_

4.6  
Full name of  
Fourth Inventor

Linda TAYLOR

Inventor's signature



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Date of signature 12th April 2001